REMARKS

In the Office Action dated April 1, 2004, claims 1-6 are pending and under consideration. Claim 1 is objected to for certain alleged informalities. Claims 1-6 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. Claims 1-6 are further rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enabling support in the specification. Claims 1-6 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Smit et al. (U.S. Patent No. 5,976,864) in view of Nomellini et al. (*J Bacteriol* 179: 6349-6354, 1997), Ausubel et al. (*Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., 1994), and Better (U.S. Patent No. 5,851,802).

This Response addresses each of the Examiner's objections and rejections. Applicant therefore respectfully submits that the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Claim 1 is objected to for the recitation of "*C. crescentus*" and "C-terminal". It is respectfully submitted that the amendment to claim 1 has overcome the objections. As such, withdrawal of the objections to claim 1 is therefore respectfully requested.

Claims 1-6 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement. The Examiner contends that the recitation "a *C. crescentus* S-layer protein fragment of at least about 120 amino acids.....and no more than 405 amino acids of the C-terminal" constitutes new matter.

Applicant respectfully submits that the specification discloses a number of fragments that contain at least about 120 amino acids and no more than 405 amino acids of the C-terminus of a *C. crescentus* S-layer protein, thereby providing support for the recitation at issue. However, in an effort to favorably advance prosecution of the present application, Applicant has

deleted the recitation in question from claim 1, rendering the rejection thereof moot. Claim 1 presently recites "a *Caulobacter crescentus* S-layer protein fragment which includes a secretion signal", which finds support throughout the specification, e.g., at page 6, line 29. Therefore, withdrawal of the rejection of claims 1-6 under 35 U.S.C. §112, first paragraph, for allegedly containing new matter, is therefore respectfully requested.

Claims 1-6 are further rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the written description requirement.

The Examiner contends that the specification only discloses a single representative species (i.e., SEQ ID NO: 5) of the recited genus *C. crescentus* S-layer proteins. The Examiner also contends that the genus of *C. crescentus* S-layer proteins encompasses species that are widely variant in their structures. As such, the Examiner is of the opinion that the disclosure of the single representative species is insufficient to be representative of the attributes and features of all species encompassed by the claimed genus. Furthermore, the Examiner states that the specification fails to provide those characteristics that distinguish the subgenus of "*C. crescentus* S-layer proteins" from the larger genus of S-layer proteins that includes S-layer proteins from *C. crescentus* and from other *Caulobacter* species.

Applicant respectfully submits that claims 1-6 are directed to methods of cleaving an insoluble fusion protein which comprises a *Caulobacter crescentus* S-layer protein fragment including a secretion signal and a protein heterologous to the *Caulobacter crescentus*. With respect to the recited element "*Caulobacter crescentus* S-layer protein fragment", the specification provides at least five examples of a *Caulobacter crescentus* S-layer protein fragment that includes a secretion signal. See, e.g., amino acids 622-1026, 690-1026, 784-1026,

784-1026, 892-1026 and 907-1026 of SEQ ID NO: 5, as disclosed on page 9, line 22; page 15, line 31; and page 17, line 26.

Although it is recognized that S-layer proteins from different strains of *Caulobacter crescentus* may differ in their amino acid sequences, the Examiner has not provided any evidence to support a notion that the S-layer proteins are so "widely variant" such that the examples provided in the specification are not representative of an S-layer protein fragment that includes a secretion signal *for purpose of practicing the claimed methods*. In this connection, Applicant respectfully submits that the present invention is not *per se* directed towards identification of *Caulobacter* S-layer protein fragments that include a secretion signal. Methods for identifying the portion of the C-terminus of a particular *Caulobacter* S-layer protein were already described to those skilled in the art at the time when the present application was filed. See, e.g., WO 97/34000. Rather, a principal feature of the present application resides in the recognition that an insoluble fusion protein can be cleaved in an acid solution, yet the S-layer protein fragment remains insoluble after cleavage thereby permitting convenient separation of the heterologous protein from the insoluble S-layer protein fragment. Based on the disclosure of the present application and the information available to those skilled in the art, it is believed that the examples of *Caulobacter cresentus* S-layer protein fragments provided in the specification are representative for a *Caulobacter cresentus* S-layer protein fragment that includes a secretion signal *for purpose of practicing the claimed methods*.

In view of the foregoing, it is respectfully submitted that the rejection of claims 1-6 under the written description requirement of 35 U.S.C. §112, first paragraph, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-6 are further rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enabling support in the specification.

The Examiner admits that the specification is enabling for a method of cleaving an insoluble fusion protein comprising a first component having amino acids 622-1026, 690-1026, 784-1026, 892-1026 or 907-1026 of SEQ ID NO: 5 and a second component that is heterologous to *C. crescentus*. However, the Examiner is concerned with the scope of the claims, stating that the claims encompass any C-terminal fragment from a *C. crescentus* microorganism, including those S-layer proteins from *C. crescentus* microorganisms yet to be isolated and *C. crescentus* mutant strains. The Examiner contends that it is highly unpredictable as to whether such S-layer proteins would maintain the same characteristics as those fragments disclosed as working examples.

Applicant respectfully submits that methods for identifying the portion of the C-terminus of a particular *Caulobacter* S-layer protein are known and routine to those skilled in the art. See, e.g., WO 97/34000. Therefore, by using routine techniques and without undue experimentation, those skilled in the art would be able to determine the portion of an S-layer protein that includes a secretion signal. Given the unique recognition that an insoluble fusion protein can be cleaved in acid solutions and a number of specific examples of fusion proteins, those skilled in the art would be able to practice the claimed methods with any fragment of an *Caulobacter crescentus* S-layer protein that includes a secretion signal.

Accordingly, it is respectfully submitted that the rejection of claims 1-6 under the enablement requirement of 35 U.S.C. §112, first paragraph, is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 1-6 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Smit et al. (U.S. Patent No. 5,976,864) in view of Nomellini et al. (*J. Bacteriol* 179: 6349-6354, 1997), Ausubel et al. (*Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., 1994), and Better (U.S. Patent No. 5,851,802).

The Examiner has characterized the teaching of Smit et al. in a previous Office Action. The Examiner contends that Smit et al. teach expression and secretion of a *Caulobacter crescentus* S-layer protein, fused to a heterologous protein. Smit et al. also teach the use of *Caulobacter* strains which shed the S-layer protein upon secretion and do not form an intact S-layer. Smit et al. disclose an example of using a shedding mutant for expression of a fusion protein that has the ability to precipitate in culture medium. The Examiner admits that the fusion protein of Smit et al. does not contain an aspartate-proline dipeptide for cleavage under acidic conditions.

The Examiner contends that Nomellini et al. teach that RsaA (S-layer protein) can be expressed as a precipitated protein from a shedding mutant of *Caulobacter crescentus*, and the precipitated protein is resistant to "low-pH solubilization".

The Examiner also contends that acid hydrolysis of a fusion protein at an aspartyl-prolyl bond was well known in the art at the time the present application was filed, as evidenced by Ausubel et al. and Better. Thus, the Examiner concludes that, based on the cited references, those skilled in the art would have been motivated to achieve the claimed methods and would have had a reasonable expectation of success in doing so.

In the first instance, Applicant respectfully submits that the rejection of claimed subject matter in view of a combination of prior art references requires that the suggestion to carry out the claimed invention must be found in the prior art, *not in Applicant's disclosure.* In

re Vaeck, 947 F.2d 488, 492, 20 U.S.P.Q. 1438, 1442 (Fed. Cir. 1991). The suggestion to include an aspartate-proline cleavage site in a fusion protein between a *Caulobacter crescentus* S-layer protein secretion fragment and a heterologous protein does not appear anywhere in the cited combination of prior art references. Therefore, the cited references have not provided any motivation to those skilled in the art to attempt to achieve the claimed methods.

Assuming, *pro arguendo*, that the references in combination had provided a motivation to achieve the claimed methods, Applicant respectfully submits that those skilled in the art would not have had a reasonable expectation that the claimed methods would be successful. In the first instance, although Nomellini et al. teach that a full-length S-layer protein produced from a shedding mutant of *Caulobacter crescentus* is precipitated and is resistant to "low-pH solubilization", Nomellini et al. do not teach a fragment of an S-layer protein that simply includes a secretion signal would also be insoluble in acid solutions. Further, there is no teaching or suggestion in Nomellini et al. that an S-layer protein fragment would remain insoluble in the pH range specifically recited, e.g., in claims 3-4.

Moreover, those skilled in the art would not have expected an insoluble protein to be successfully or efficiently cleaved at an aspartyl-prolyl bond. As described in the Background section of the specification (page 5, lines 24-30), prior to the present invention, the protein to be cleaved was typically exposed to conditions that solubilize or completely denature the protein prior to cleavage. Therefore, there is nothing in any of the cited references that would have provided to those skilled in the art a reasonable expectation that an insoluble fusion protein aggregate would be sufficiently exposed for successful cleavage at an aspartyl-prolyl bond. For the same reason, those skilled in the art would not even have been motivated to attempt to place an aspartyl-prolyl bond in an insoluble protein, as presently claimed.

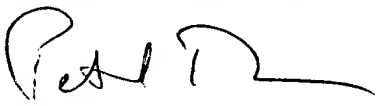
In contrast, the present application uniquely identified that insoluble fusion proteins can be cleaved under acidic conditions that permit cleavage at the aspartyl-prolyl bond on one hand, and that do not solubilize the fusion protein on the other hand. In addition, the resulting S-layer fragment remains insoluble in the acidic solution after cleavage, thereby permitting easy separation of desired protein product.

Applicant respectfully submits that the cited references, taken alone or in combination, do not provide those skilled in the art with a motivation or a reasonable expectation of success to try to arrive at the claimed invention. Thus, the rejection of claims 1-6 under 35 U.S.C. §103(a) in view of the combination of the references is overcome. Withdrawal of the rejection is therefore respectfully requested.

Claims 9-12 are added and are supported by claims 1-6 and the specification. No new matter is introduced.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,



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